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Neonatal Triethyltin Exposure Alters Adult Electrophysiology in Rats

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16. ABSTRACT

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NEONATAL TRIETHYLTIN EXPOSURE ALTERS ADULT ELECTROPHYSIOLOGY IN RATS[1]

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In adults, triethyltin (TET) produces degeneration of white matter, edema, vacuolization of myelin and histotoxic hypoxia. To determine the functional consequences of perinatal exposure to TET, albino rats were administered either 0, 3, 6, or 9 mg/kg TET on postnatal day 5. Upon reaching adulthood, the rats were implanted with electrodes for recording visual evoked potentials (VEPs) and hippocampal afterdischarges (ADs). In addition to these tests, 17 days of kindling trials were administered to the rats followed by testing with pentylenetetrazol and picrotoxin for seizure susceptibility. TET increased latencies of P2, P3, and N3 of the VEP in a dose dependent fashion. TET also decreased N1P2 amplitudes and produced gender-specific alterations in both P1, N1, and N2 latencies and N2P3 amplitudes. TET produced alterations in duration of the AD recorded from cortex during kindling, but did not produce significant alterations in any of the other variables tested. The results support previous studies, since they show that the adult VEP is sensitive to perinatal toxicant exposure.

INTRODUCTION

In adults, triethyltin (TET) produces degeneration of white matter, owing to edema and vacuolation of myelin (Aleu *et al.*, 1963). In addition, TET produces a histotoxic hypoxia by inhibiting mitochondrial respiration (Aldridge and Rose, 1969). In adults, it is difficult to separate the hypoxic and myelinopathic consequences of exposure to TET. However, if exposure occurs at a developmental stage prior to the onset of myelination, then consequences of the exposure may be attributed more readily to the hypoxic effects of the substance, although such exposures nevertheless retard development of full myelination (Wender *et al.*, 1974).

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[1]This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Considerable evidence suggests that the developing organism is sensitive to neurotoxic insult (e.g., Dyer *et al.*, 1978; Lochry and Riley, 1980; Fechter and Annau, 1977). In the present experiment, rats were exposed to TET on postnatal day 5 before the beginning of myelination (Wender *et al.*, 1974). This paradigm which has been successfully explored by Reiter *et al.* (1980) exposes the rat while the brain is rapidly developing, yet bypasses many of the problems attendant to prenatal exposures.

Electrophysiological techniques allow assessment of the integrity of different functional units within the CNS. The visual evoked potential (VEP) has frequently been used as an electrophysiological probe of the functional integrity of the visual system (e.g., Dyer, 1980). Because the optic nerve was among the structures damaged following exposure of adults to TET (Scheinberg *et al.*, 1966), and because the VEP is under investigation in our laboratory as a general screen for potential neurotoxicants, animals exposed to TET on postnatal day 5 were subjected to a VEP test as adults.

Various seizure models have also been proposed as potentially useful in assessing neurotoxicity (Dyer *et al.*, 1979b; Fox *et al.*, 1979). To explore the value of seizure models in assessment of neurotoxicity induced by perinatal exposure to TET, one electrical (hippocampal afterdischarge) and two pharmacological (picrotoxin and pentylenetetrazol) models were used in this study. The main goals of the project were to determine (1) which of these neurophysiological tests provide the most sensitive index of exposure to TET (2) which measures making up each test are most useful in assessing the consequences of the exposure, and (3) some of the functional implications of the exposure.

MATERIALS AND METHODS

Animals

Pregnant CD rats (Charles River, Wilmington, MA) were obtained 2 days after mating and housed singly in cages measuring 45 x 24 x 20 cm. On the day of parturition (day 0), pups from 10 litters were pooled and randomly reassigned to form litters of 8 pups (4 males, 4 females). TET bromide (ALFA Products, Danver, MA) was dissolved in sterile saline just prior to treatment; concentration was adjusted so that each pup received an injection volume of 10 μl/gm body weight. On day 5, pups were injected ip with either 0,3,6, or 9 mg/kg TET. A within-litters design was used for dosing: one female and one male from each litter received each dose, therefore, each litter contained all treatments in both sexes. At 21 days of age, animals were weaned and littermates were housed by sex in groups of 3 or 4 in cages of the same size. The animal room was mantained on a 12 hr light-dark cycle beginning at 0600 hr, and food (Purina Lab Chow) and water were available *ad libitum* throughout the experiment. At 60 days of age, electrodes were surgically implanted and animals were placed into individual cages. Mortality data obtained from these animals will be presented elsewhere (Reiter *et al.*, 1981).

Surgery

Animals were anesthetized with 3.5 ml/kg Chloropent (Fort Dodge Laboratories, Inc.). Stainless steel screws (00-90x1/16") were threaded into the skull 5 mm anterior to bregma and 2 mm lateral for ground (left side) and reference (right side) electrodes. The visual cortex electrode was a screw placed 6 mm posterior to bregma and 3 mm lateral to the midline. Two sets of bipolar electrodes were implanted into the hippocampus. Both sets were constructed of twisted stainless steel teflon-coated .005" wire. Stereotaxic placements were made with the incisor bar 5 mm above the interaural line. The first set was aimed at the dentate gyrus and was placed 2.5 mm posterior to bregma, 2.5 mm lateral to the midline, and 3.0 mm below the cortical surface. The second set of hippocampal electrodes, aimed at the CA1 cell field, was placed immediately anterior and about 0.75 mm dorsal to the dentate gyrus set. All electrodes were connected to an Amphenol receptacle which was subsequently cemented to the skull with dental acrylic. Following surgery, each animal received 100,000 units Penicillin G (im).

Testing Sequence

Following surgery, at least 10 days elapsed before testing was begun. The following sequence of tests was performed: on the first test day VEPs were recorded followed immediately by afterdischarge (AD) tests; animals were then given 17 consecutive days of kindling; 3-6 days later, pentylenetetrazol-induced seizures were evaluated; and finally, 6-9 days later picrotoxin-induced seizures were studied. At the end of these studies, all animals were perfused with saline followed by formalin. Brains were removed, sectioned in the sagittal plane, stained with cresyl violet, and examined for histological verification of electrode placements.

Visual Evoked Potentials

To ensure pupillary dilation (and thereby eliminate variation in VEP parameters due to variations in pupil size), a drop of 0.1% (w/v) atropine sulfate was placed upon each eye 15 min prior to testing. At the time of testing, each animal was connected via a recording cable to the amplifier (high and low frequency cut offs for the amplifier were 10 khz and 0.1 hz, respectively) and placed in the recording chamber. Details of the recording chamber have been provided elsewhere (Dyer and Annau, 1977). Briefly, it was a rectangular box with mirrors on three sides and the lamp face of a Grass PS-2 photostimulator mounted against the fourth. Each animal was given a 5 min habituation period of 0.5 hz flashes before data collection was begun. Following this, a series of 4 averages (64 flashes each) was obtained at each of four flash intensities, presented in descending order. These intensities were estimated by an EG&G photometer/integrator to provide peak power of 4.53×10^7, 8.46×10^6, 4.86×10^6, and 2.1×10^6 Lux, respectively, and corresponded to settings 16, 4, 2, and 1 on the photostimulator. Latencies and amplitudes for the major peaks of the VEP were determined by a Nicolet signal averager. Latencies were recorded to

the nearest 0.4 msec, the sampling rate of the averager was 0.4 msec/pt, for 1024 pts. Each averaged evoked potential was also plotted by an X-Y recorder.

Hippocampal Afterdischarges

Three ADs were produced in each animal during each test session. The first afterdischarge (AD1) was used to determine the stimulus threshold for AD production. Each animal was stimulated once/min through the bipolar dentate gyrus electrodes with an ascending series of stimulus intensities (20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, and 200 uA) until an AD occurred. Each stimulus period consisted of a 2 sec train of 50 hz pulses. Each pulse was a biphasic square wave with 0.2 msec/phase. Fifteen min after the end of AD1, each animal was given a supramaximal stimulus (4 x threshold) to produce AD2. The purpose of AD2 was to ensure that animals had relatively consistent types of ADs, so that their properties could be scored (Dyer *et al.*, 1979b). Ten min after the end of AD2, stimulation was begun to elicit AD3. The purpose of AD3 was to determine the time course of postictal hypoexcitability (Swartzwelder *et al.*, 1980). Stimuli occurred once every 2 min at twice threshold until AD3 was elicited.

For AD1, the threshold, type and duration (in dentate gyrus) were scored. Four types of hippocampal ADs have been described (Dyer *et al.*, 1979b): type 1 consists of an AD followed by a depression which is interrupted by a brief rebound AD; type 1a is different from type 1 in that no rebound AD occurs; type 2 consists of an AD followed by no depression, but with irregular spikes occurring during the postictal period; type 2a differs from type 2 in that no postictal spikes occur.

For AD2, the following variables were scored: type; duration (in cortex, dentate gyrus and CA1); number of wet dog shakes (WDS) (Dyer *et al.*, 1979b); EEG spike frequency (in three separate 3-sec blocks: beginning, middle, and end of AD); and postictal depression (PID) magnitude. PID magnitude was estimated by connecting the amplified EEG to an integrator. The sensitivity of the integrator was adjusted so that during a period of normal EEG (i.e., before stimulation), it reached its maximum and reset about 10 times/min. The number of resets occurring for 5 consecutive min following the AD was then determined. In order to adjust for individual differences in prestimulation EEG resets, each min of post AD activity resets was divided by the prestimulation value.

The variables recorded for AD3 were: number of stimuli until AD3 was elicited (a minimum duration of 5 sec was required for a given AD to be counted); AD type; and AD duration.

Kindling

The kindling experiment required daily stimulation for 17 consecutive days at a stimulus intensity of 4 times AD threshold. During each test, ADs were recorded from dentate gyrus, CA1, and cortex and the rat's behavior was observed. Behavioral seizures were rated on a 5 point scale (Racine, 1972): (1) facial movements; (2) head nodding; (3) forelimb clonus; (4) rearing; and (5)

rearing and falling. The dependent variables were seizure score and AD duration. In order to reduce the number of dependent variables, only the scores for days 1, 2, 5, 8, 11, 14, and 17 were used.

Pentylenetetrazol

During pentylenetetrazol (metrazol) testing, the EEG was recorded from the cortex. Pentylenetetrazol was administered sc at 70 mg/kg and the following dependent variables were scored: latency to first twitch; latency to clonus, latency to end of first seizure episode; latency to first burst of EEG spikes; number of EEG bursts in the 60 sec after the first burst; and number of EEG bursts 60 sec prior to the behavioral seizure.

Picrotoxin

Picrotoxin was given in a dosage of 2 mg/kg ip and the following measurements were made: latency to first twitch, latency to clonus, latency to end of the first episode, seizure score (using the 5 point rating scale used in the kindling experiment); and time from clonus to the end of the first episode.

Number of Subjects/Experiment

All of the experiments reported here were performed on the same population of animals. Although all animals were implanted with the same electrode arrays, not all of the recordings were equally satisfactory (i.e., some were electrically noisy). Thus, some animals with satisfactory visual cortex recordings did not have satisfactory hippocampal recordings, etc. In addition, as the experiment proceeded, some animals either pulled their head plugs or died during a seizure. These animals obviously were excluded from the rest of the studies, but their data were included for the studies they had completed. Table 1 shows the distribution of subjects by dosage and gender for the different studies reported here.

TABLE 1. Distribution of Subjects by Dosage, Gender, and Experiment

TET Dosage (mg/kg)	Gender	Total Implanted	Visual Evoked Potential	After-discharge	Kindling	Metrazol	Picrotoxin
0	male	8	7	7	7	7	8
	female	8	6	6	6	3	3
3	male	8	8	7	7	6	5
	female	9	9	7	7	5	5
6	male	5	4	5	5	5	3
	female	7	6	7	7	6	6
9	male	3	3	2	2	1	1
	female	5	5	5	5	4	4

Statistical Analysis

The visual evoked potential data were analyzed using multivariate statistical techniques (MANOVA). The six latencies (P1, N1, P2, N2, P3, and N3) and five peak-to-peak amplitudes (P1N1, N1P2, P2N2, N2P3, and P3N3) obtained under each of the 4 stimulus intensities were the dependent variables. Separate MANOVAs were performed using the general linear models procedure (GLM) of SAS79 (Helwig and Council, 1979) to determine overall effects of treatment, gender, intensity of flash, gender x treatment interactions, intensity x dosage interactions, and gender x intensity x dosage interactions. A Bonferroni correction was used to adjust the required significance level for each MANOVA to compensate for the fact that 6 MANOVAs were done (.05/6 = .0083). If overall MANOVAs were significant, univariate ANOVAs were performed, which, if significant, were followed by Duncan's Multiple Range Test. This same analysis strategy was adopted for all of the other studies. To ensure an overall α level of .05, the Bonferroni correction was also applied to ANOVAs. Since 11 dependent variables were measured in the VEP study (6 latencies, 5 amplitudes) any given univariate ANOVA was required to have a $p \leq .05/11$ (.0045) in order to be considered statistically significant.

RESULTS

VEP

Of the different tests performed upon the TET-treated animals, the one which clearly differentiated among the different exposure groups was the VEP. The results of the MANOVA and ANOVA (when appropriate) are indicated in Table 2.

TABLE 2. Statistical Summary for Day 5 TET Exposure Visual Evoked Potential Study

		Latency ANOVAs						Amplitude ANOVAs				
Source	MANOVA[1]	P1	N1	P2	N2	P3	N3	P1N1	N1P2	P2N2	N2P3	P3N3
Dosage	p<.0001	.0651	.1800	.0001[2]	.0001[2]	.0001[2]	.0001[2]	.209	.0035[2]	.157	.0001[2]	.0609
Gender	p<.0001	.0016[2]	.0112	.70	.55	.205	.0013[2]	.0001[2]	.0001[2]	.0065	.0001[2]	.0001[2]
Intensity	p<.0001	.0001[2]	.0001[2]	.99	.96	.117	.501	.0001[2]	.0001[2]	.512	.906	.0079
Dosage x Gender	p<.0001	.0043[2]	.0009[2]	.063	.0001[2]	.54	.227	.0501	.0163	.0839	.0017[2]	.0391
Dosage x Intensity	p<1.000	—	—	—	—	—	—	—	—	—	—	—
Dosage x Gender x Intensity	p<1.000	—	—	—	—	—	—	—	—	—	—	—

[1]Using exact multivariate F (see Rao, 1965).
[2]Considered significant after Bonferroni correction (critical value = .0045).

Treatment In general, TET increased latencies in a dosage-dependent fashion which was most pronounced in the later peaks (N2, P3, N3). TET decreased N1P2 amplitude, but increased N2P3 amplitude. The MANOVA for dosage was significant [$F(33,589) = 8.70$, $p < .0001$]. ANOVA indicated that the dependent variables which were significantly influenced by TET (after the Bonferroni correction) were: P2; N2; P3; and N3 latencies; and N1P2 and N2P3 amplitudes [all F's$(3,210) > 4.70$, all p's $< .0035$]. P2 and N2 (see Figs. 1 and 3) latencies increased at 3 and 6 mg/kg. Although latencies of these two peaks were also above controls at 9 mg/kg, they were below those at 6 mg/kg. Duncan's Multiple Range Test ($\alpha = .05$), used to make comparisons between dosages, indicated that for P2 latency the 6 mg/kg group was significantly different from all other groups. No other dosages significantly altered P2 latency. The presence of an increased P2 latency in the absence of alterations in P1 and N1 latencies implies a broadening of the P1N1P2 wave. To test this hypothesis, an ANOVA was performed upon the P2-P1 latency differences. The results supported the hypothesis [$F(3,246) = 16.56$, $p < .0001$], and the Duncan's Multiple Range Test indicated that the results were accounted for by the 6 mg/kg group which was significantly different from all others. For N2 latency, the 0 mg/kg group was significantly different from all other groups, and the 6 mg/kg group was significantly different from the 3 and 9 mg/kg groups. Increasing dosage of TET also increased peak latency of P3 and N3. In the case of P3, the 0 mg/kg group was significantly different from all others, as was the 3 mg/kg group. The function appears to asymptote at 6 mg/kg. In the case of N3, all groups were significantly different from each other. Figure 1 shows the influence of dosage upon the latencies of P2, P3 and N3.

With respect to amplitudes, the Duncan Multiple Range Test indicated that only the 9 mg/kg group had N1P2 amplitudes which significantly differed from any other group, and these were different from all other groups. Similarly, the N2P3 amplitudes were only significantly affected by the highest dosages of TET. The N2P3 amplitudes of the 9 mg/kg group were nearly twice as large as those of any other group (see Fig. 3). Figure 2 illustrates the influence of TET dosage upon N1P2 amplitudes.

To further characterize the effects of the day 5 TET exposure upon adult VEPs, a trend analysis was performed upon those variables which were significantly affected by TET. This analysis is summarized in Table 3. Both the P3 and N3 latency functions are best described by a linear trend although the P3 latency function has a significant quadratic trend as well. The P2 latency had significant linear, quadratic, and cubic trends.

Gender In general, females had higher amplitudes than males. Females also had shorter P1 latencies and longer N3 latencies. The MANOVA for gender was significant [$F(11,200) = 7.34$, $p < .0001$]. ANOVAs indicated that the dependent variables which were significantly influenced by gender (after the Bonferroni correction) were: P1 and N3 latencies; and P1N1, N1P2, N2P3 and P3N3 amplitudes [all F's $(1,210) \geq 10.27$, all p's $< .0016$]. Table 4 summarizes these results. A separate MANOVA indicated that there was a significant treatment x gender interaction [$F(33,589) = 2.76$, $p < .0001$].

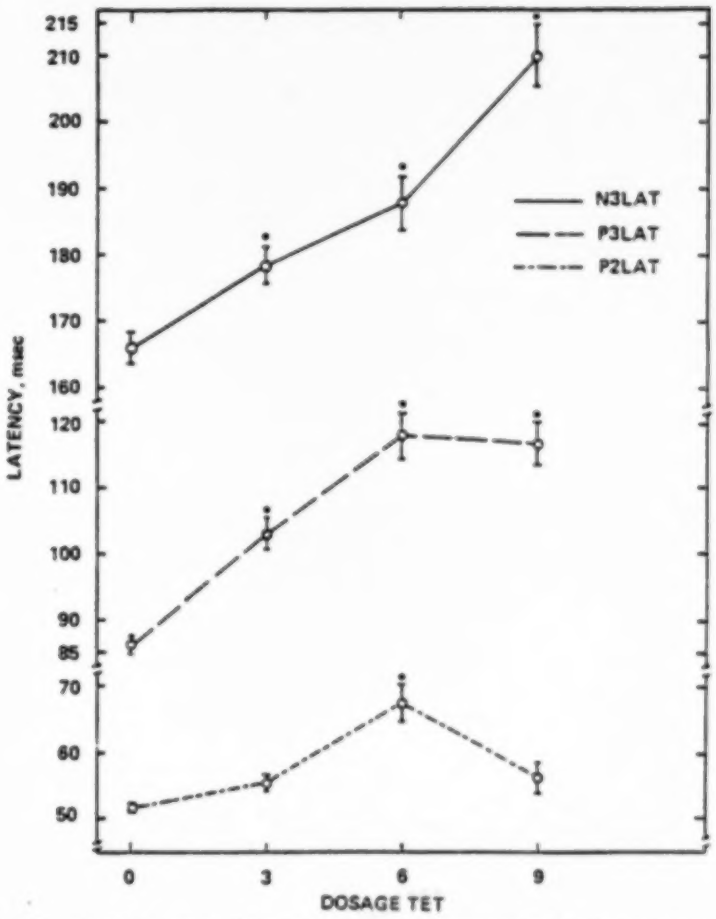

FIGURE 1. Influence of day 5 postnatal exposures to TET upon mean (± SEM) VEP latencies recorded from adult unanesthetized rats. Only those peaks for which the main effect of dosage was significant and no dosage x gender interaction was significant are plotted. * = Different from control using Duncan's Multiple Range Test ($p < .05$).

ANOVAs indicated that the dependent variables which were significantly influenced by the treatment x gender interaction were: P1; N1; and N2 latencies; and N2P3 amplitudes. Figure 3 illustrates the treatment x gender interaction for the P1, N1 and N2 latencies and the N2P3 amplitudes. The Duncan Multiple Range Test indicated that for both P1 and N1 latencies, only the 9 mg/kg male group differed significantly from its same gender control. With respect to the N2 latencies, only the 9 mg/kg female group did *not* differ significantly from its same gender control. N2P3 amplitudes were significantly increased at 9 mg/kg for females, whereas they were significantly increased at 3, 6, and 9 mg/kg for males.

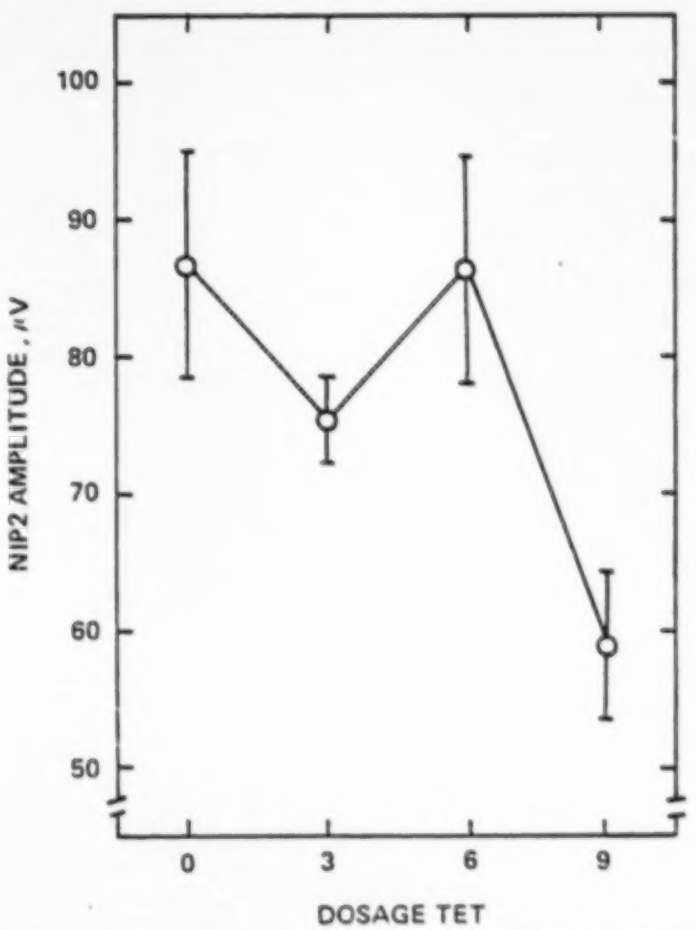

FIGURE 2. Influence of day 5 postnatal exposures to TET upon mean (± SEM) VEP amplitude of N1P2 peak recorded from adult unanesthetized rats. N1P2 was the only amplitude for which the main effect of dosage was significant and no dosage x gender interaction was significant. The 9 mg/kg group differed significantly from all others using Duncan's Multiple Range Test ($p < .05$).

TABLE 3. Trend Analysis of VEP Components Influenced by TET[1]

		P2	P3	N3	N1P2
Linear:	F =	10.38[2]	77.57[4]	79.20[4]	5.50
Quadratic:	F =	19.80[4]	12.02[3]	2.09	1.48
Cubic:	F =	18.46[4]	1.68	1.23	4.66

[1]All df's = 1,246.
[2]$p < .01$.
[3]$p < .001$.
[4]$p < .0001$.

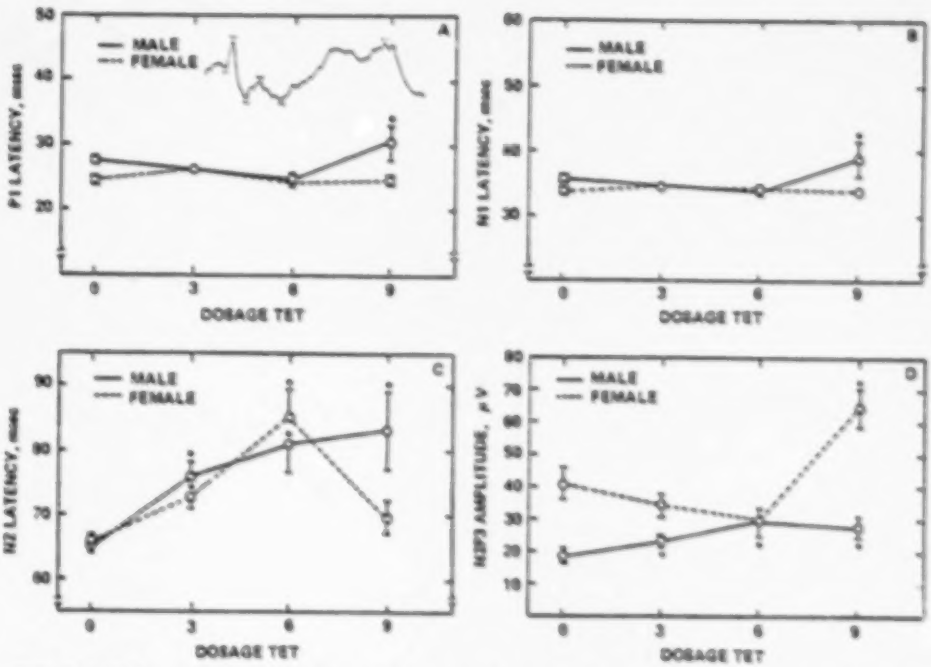

FIGURE 3. Differential effects of day 5 postnatal exposures to TET upon mean (± SEM) latency and amplitude components of the VEP recorded from adult unanesthetized male and female rats. A: P1 latency, B: N1 latency; C: N2 latency, and D: N2P3 amplitude. * = Different from same gender 0 mg/kg TET control using Duncan's Multiple Range Test ($p < .05$).

TABLE 4. Mean Amplitudes and Latencies (±SEM) for Peaks with Significant Gender Differences[1]

	Latencies (mSec)		Amplitudes (μV)			
Gender	P1	N3	P1N1	N1P2	N2P3	P3N3
Female	25.1±0.3	186.6±2.4	57.2±3.3	91.8±5.0	40.4±2.4	78.3±5.1
Male	26.9±0.5	176.3±2.8	38.1±2.0	60.8±2.7	23.4±1.4	49.1±2.2

[1]All tabled gender comparisons differed significantly (all p's < .0016). Values based on all males and all females.

Intensity In general, increasing the flash intensity produced decreased latencies and increased amplitudes. The MANOVA for intensity was significant [$F(44,767 = 2.81)$, $p < .0001$]. Following Bonferroni correction, the following dependent variables were indicated to have been significantly altered by the intensity variable: P1, and N1 latencies; and P1N1 and N1P2 amplitudes. However, there was not a significant treatment x intensity interaction [$F(132,1653) = 0.53$, $p < 1.00$] nor was there a significant treatment x gender x intensity interaction [$F(132,1653) = 0.55$, $p < 1.00$].

Hippocampal ADs

MANOVAs indicated that there was no significant effect of gender [$F(13,25) = 0.32$, $p < .99$], treatment [$F(39,81) = 0.75$, $p < 0.84$], or gender x treatment [$F(39,81) = 0.81$, $p < 0.76$].

Hippocampal Kindling

MANOVAs indicated that there were overall significant effects of treatment, gender, stimulation number (i.e., day), and treatment x gender. These MANOVAs and the ANOVAs which followed are summarized in Table 5.

TABLE 5. Statistical Summary for Day 5 TET Exposure Hippocampal Kindling Study

		ANOVA's			
Source	MANOVA[1]	Seizure score	Cortex AD duration	CA1 AD duration	DG AD duration
Dosage	p<.0001	0.0346	0.0003[2]	0.0591	0.9865
Gender	p<.0004	0.0523	0.7825	0.1469	0.1483
Day	p<.0285	—	—	—	—
Dosage x Gender	p<.0001	0.1289	0.0010[2]	0.3865	0.0084[2]
Dosage x Day	p<.9991	—	—	—	—

[1]Using Exact multivariate F (see Rao, 1965).
[2]Considered significant after Bonferroni correction (critical value = .0125).

Dosage of TET significantly altered only cortical AD duration. There were no significant treatment x day interactions, but the treatment x gender interaction was significant for cortex AD duration and dentate gyrus AD duration. Day of testing did not significantly alter any seizure variables. These results are summarized in Fig. 4.

Pentylenetetrazol

MANOVAs indicated that there was no overall effect of treatment [$F(27,444) = 0.93$, $p < .57$], nor was there a significant treatment x gender interaction [$F(27,44) = 1.38$, $p > .168$].

Picrotoxin

MANOVAs indicated that there was no overall effect of treatment [$F(9,53) = 1.05$, $p > .414$], nor was there a significant treatment x gender interaction [$F(9,53) = 1.38$, $p > .221$].

Histology

Examination of electrode placements revealed that all depth electrodes were appropriately placed.

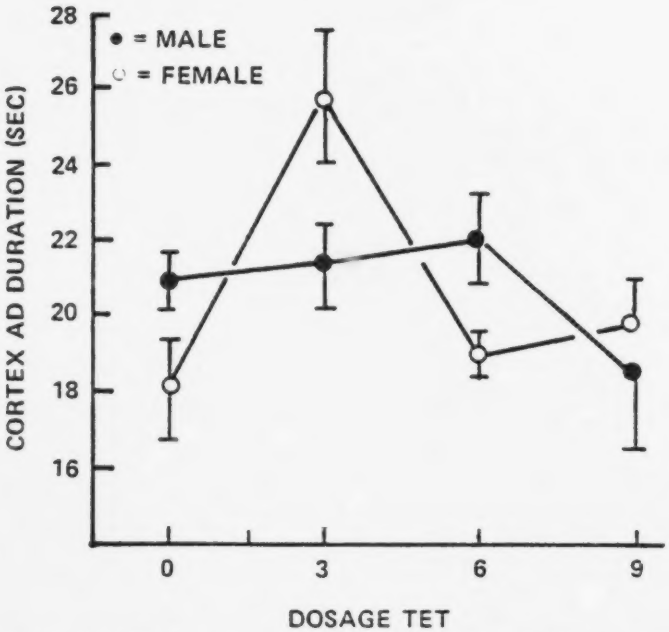

FIGURE 4. Summary of kindling experiment. Females exposed to the 3 mg/kg dosage of TET had significantly longer cortical ADs than all other groups.

DISCUSSION

Only the VEP technique detected any clear influence of the perinatal exposure to TET. Considering how insensitive the other techniques were, it was surprising to find that the VEP technique demonstrated treatment-related differences on so many of the dependent variables. The pattern of alterations in the VEP allows some inferences to be made about the nature of TET-induced alterations in CNS function. The characteristics of P1 and N1 are generally taken to reflect input to the cortex via the retinogeniculate pathway, whereas later peaks are recognized as reflecting nonspecific or recurrent activity (Creel *et al.*, 1974). Therefore, increased latencies of peaks P2, P3, and N3 at 6 mg/kg TET, in the absence of significant increases in latency of peaks P1 and N1, suggest that TET exposure did not affect the retina, optic tract, or lateral geniculate body. The failure to find alterations in P1N1 amplitude at any dosage and in N1P2 amplitude below the 9 mg/kg dosage provides support for ruling out these noncortical structures as target loci for the lower dosage level effects. It thus seems likely that in the present experiment, the conduction or synaptic properties of either the visual cortex or nonspecific inputs to the visual cortex have been altered. These results are very different from those obtained when adult rats are given 7 daily exposures to TET. In that case, the optic nerve is presumed to be involved, and the main effect observed is increased latency of all VEP peaks (Howell and Dyer, 1980).

Some of the effects of TET upon the VEP were gender-dependent, including the P1, N1, and N2 latencies and N2P3 amplitudes. In most cases, the effects of TET on the two genders were similar at dosages of 0, 3, and 6 mg/kg. At 9 mg/kg, the males had significantly increased P1 and N1 latencies. Thus, males appear more sensitive to whatever process increases P1 and N1 latencies at high dosages of TET than do females.

In contrast, the gender x dosage interactions for N2 latency and N2P3 amplitude appear to result from uncharacteristically low and high readings, respectively, for the females at the 9 mg/kg dosage. N2 latencies increased for both males and females at 3 and 6 mg/kg TET, and although they remained elevated at 9 mg/kg TET in males, the females' N2 latencies returned to control levels at the 9 mg/kg dosage. Effects of TET on N2P3 amplitudes were somewhat more complex, but the major difference is the substantial increase observed in the females given 9 mg/kg TET. The fact that most of the gender x dosage interactions are easily accounted for by values at the 9 mg/kg dosage, coupled with the small sample size in the 9 mg/kg dosage (3 male, 5 female), could indicate that a few aberrant animals contributed to the findings. These findings should be replicated before they are extensively discussed.

These results point out the very different consequences for adult electrophysiology of hypoxia produced by prenatal low level carbon monoxide (CO) exposure and hypoxia produced by early postnatal TET exposure (via uncoupling of oxidative phosphorylation). Continuous gestational exposure to 150 ppm CO (maternal HbCO = 15%) has been shown to increase the amplitude of P1N1 and N1P2 amplitudes, but only in females (Dyer *et al.*, 1979a). No other amplitudes were affected by the exposure nor were any latencies affected. The qualitative differences between the CO experiment and the present TET experiment may result from either the time at which the exposures were made, the cellular mechanism by which hypoxia was produced, or an effect of TET unrelated to hypoxia. For the following reasons, it is unlikely that exposure level (i.e., extent of hypoxia) accounts for the difference. The only variable which was affected by both procedures, N1P2 amplitudes, was affected only at high dosages of TET (9 mg/kg), and at this point the amplitudes were decreased, whereas CO increased the amplitude of this peak. It cannot be argued that the 9 mg/kg TET exposure was below the equivalent level of CO exposure in severity since none of the other variables affected by TET were also affected by CO exposure. If the CO exposure was more severe than the TET exposure, then every variable affected by TET should have been affected to an even greater extent by exposure to CO.

These data suggest that visual function is impaired by the day 5 exposure to TET. The nature of this impairment is not directly discernible from these data, but the pattern of increased latencies in the later peaks is suggestive of slowed processing of visual information at the cortical level.

The failure of any of the seizure tests to detect TET-induced alterations in brain function was surprising. At the time of treatment (day 5 postnatal), the cells of the hippocampus are still undergoing rapid growth (Bayer, 1980). Consequently, if toxicity depends solely upon rapid growth occurring at the time of treatment, the hippocampal AD and/or kindling tests should have

produced some separation of groups. If the alterations occurred generally in cortical regions, either of the pharmacological models of seizures should have detected toxicity since these are presumed to generate seizures by acting primarily upon cortical areas (Stone, 1972).

The findings thus indicate that although TET is supposed to be a non-specific metabolic inhibitor (Aldridge and Rose, 1969), there is a regional specificity of effect produced by day 5 exposure. This regional specificity cannot be explained entirely by knowing which cells are actively undergoing development at the time of treatment since visual cortex cells are also rapidly differentiating on postnatal day 5 (Juraska and Fifková, 1979). The dimensions of the visual system damage remain to be explored with behavioral techniques.

Finally, these results demonstrate that (1) the evoked potential technique provides the most sensitive neurophysiological index of TET exposure yet tested: (2) altered latencies are most useful in assessing the consequences of day 5 TET exposure; and (3) visual function is permanently impaired following day 5 TET exposure. The extent to which brain regions other than those examined here are damaged by day 5 TET exposure remains to be determined.

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